

REPLACED BY
AMENDMENT

Claims

- 5 1. An isolated, refolded NspA protein.
2. A method for refolding an NspA protein comprising contacting the NspA protein with an alkaline refolding buffer comprising 3-dimethyldodecylammoniopropanesulfonate (SB-12).
- 10 3. A method according to claim 2 wherein the refolding buffer comprises ethanolamine and SB-12.
- 15 4. A method according to claim 3 wherein the ethanolamine is about 20mM ethanolamine.
5. A method according to any one of claims 2 to 4 wherein the refolding buffer has pH11.
- 20 6. A method according to any one of claims 2 to 5 wherein the SB-12 is 0.2% SB-12.
7. A method according to any one of claims 2 to 5 wherein the SB-12 is 0.5% SB-12.
- 25 8. A method according to any one of claims 2 to 7 wherein the SB-12 is purified.
9. A method according to claim 8 wherein the SB-12 is purified by passing it over an Al₂O₃ column.
- 30 10. A method comprising the following steps:
 - a. optionally expressing an NspA protein in a host cell;
 - b. optionally breaking the host cell to obtain an inclusion body comprising the NspA protein;
 - c. optionally washing the inclusion body;
 - d. optionally solubilisation of at least part of the inclusion body and the NspA protein;
 - 35 e. contacting the solubilised NspA protein with the refolding buffer; and

f. optionally removing the refolding buffer from the NspA protein.

11. A refolding buffer comprising ethanolamine and SB-12 for use in the method of any one of claims 2 to 10.

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12. An isolated, refolded NspA protein obtained or obtainable by the method of any one of claims 2 to 10.

10 13. A pharmaceutical composition comprising at least one isolated, refolded NspA protein of claim 1 or 12, and a pharmaceutically acceptable carrier.

14. A pharmaceutical composition according to claim 13 wherein at least 30%, 50%, 70%, or 90% of the NspA protein present in the composition is refolded.

15 15. A pharmaceutical composition according to claim 13 or 14 in the form of a vaccine.

16. The pharmaceutical composition of any one of claims 13 to 15 comprising an isolated, refolded NspA protein derived from *Neisseria meningitidis*.

20 17. The pharmaceutical composition of any one of claims 13 to 16 comprising an isolated, refolded NspA protein derived from *Neisseria gonorrhoeae*.

18. The pharmaceutical composition according to any one of claims 13 to 16 wherein said composition comprises at least one other Neisserial antigen.

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19. The pharmaceutical composition of claim 18 comprising at least one other Neisserial antigen derived from *Neisseria gonorrhoeae*.

30 20. The pharmaceutical composition of claim 18 or 19 comprising at least one other Neisserial antigen derived from *Neisseria meningitidis*.

21. A pharmaceutical composition according to any one of claims 18 to 20 further comprising at least one other Neisserial antigen selected from one or more of the following classes:

a. at least one Neisserial adhesin selected from the group consisting of FhaB, Hsf, NadA, PilC, Hap, MafA, MafB, Omp26, NMB0315, NMB0995 and NMB1119;

b. at least one Neisserial autotransporter selected from the group consisting of Hsf, Hap, IgA protease, AspA and NadA;

5 c. at least one Neisserial toxin selected from the group consisting of FrpA, FrpC, FrpA/C, VapD, NM-ADPRT, and either or both of LPS immunotype L2 and LPS immunotype L3;

d. at least one Neisserial Fe acquisition protein selected from the group consisting of TbpA high, TbpA low, TbpB high, TbpB low, LbpA, LbpB, P2086, HpuA, HpuB,

10 Lipo28, Sibp, FbpA, BfrA, BfrB, Bcp, NMB0964 and NMB0293; and

e. at least one Neisserial membrane associated protein, preferably outer membrane protein, selected from the group consisting of PldA, TspA, FhaC, NspA, TbpA(high), TbpA(low), LbpA, HpuB, TdfH, PorB, HimD, HisD, GNA1870, OstA, HlpA, MltA, NMB 1124, NMB 1162, NMB 1220, NMB 1313, NMB 1953, HtrA, TspB, PilQ and

15 OMP85.

22. The pharmaceutical composition of any one of claims 13-21 further comprising one or more bacterial capsular polysaccharides or oligosaccharides.

20 23. The pharmaceutical composition of claim 22 wherein the one or more capsular polysaccharides or oligosaccharides are derived from bacteria selected from the group consisting of *Neisseria meningitidis* serogroup A, C, Y, and/or W-135, *Haemophilus influenzae* b, *Streptococcus pneumoniae*, Group A Streptococci, Group B Streptococci, *Staphylococcus aureus* and *Staphylococcus epidermidis*, and are 25 preferably conjugated to a source of T-helper epitopes.

24. Use of an NspA protein of any one of claims 1 and 12 (or a pharmaceutical composition of claims 13-23) in the preparation of a medicament for use in generating an immune response in an animal.

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25. Use of an NspA protein of any one of claims 1 and 12 (or a pharmaceutical composition of claims 13-23) in the preparation of a medicament for treatment of prevention of Neisserial infection.

26. A method of preventing or treating Neisserial infection by administering an NspA protein of any one of claims 1 and 12 (or a pharmaceutical composition of claims 13-23) to a patient in need thereof.

5 27. The use or method of claim 25 or 26 in which *Neisseria meningitidis* infection is prevented or treated.

28. The use or method of claims 25-27 in which *Neisseria gonorrhoeae* infection is prevented or treated.

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29. An antibody immunospecific for the NspA protein as claimed in any one of claims 1 and 12.

15 30. A pharmaceutical composition useful in treating humans with a Neisserial disease comprising at least one antibody according to claim 29 and a suitable pharmaceutical carrier.

31. Use of the antibody of claim 29 in the manufacture of a medicament for the treatment or prevention of Neisserial disease.

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32. The use of claim 31 in which *Neisseria meningitidis* infection is prevented or treated.

33. The use of claim 31 or 32 in which *Neisseria gonorrhoeae* infection is prevented or treated.

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34. A method of diagnosing a Neisserial infection, comprising the steps of identifying an NspA protein, or an antibody thereto, within a biological sample from an animal suspected of having such an infection using an NspA protein as claimed in any one of claims 1 and 12, or an antibody as claimed in claim 29.

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35. The method of claim 34 in which *Neisseria meningitidis* infection is diagnosed.

36. The method of claim 34 or 35 in which *Neisseria gonorrhoeae* infection is diagnosed.